What is claimed is:

1. A method of identifying and selecting transformants comprising;

transforming a host cell with *Agrobacterium* under suitable conditions whereby recombination occurs, the *Agrobacterium* comprising a vector containing a targeting construct wherein said construct comprises a first polynucleotide sequence encoding a negative selection marker linked to a fragment of DNA flanked by DNA sequences homologous to a polynucleotide to be targeted, wherein said DNA fragment is disrupted by a positive selection marker; and

selecting transformants by subjecting a transformed host cell to a positive and a negative selection agent.

2. The method of claim 1, wherein transformants resulting from a knockout lack a negative selection marker and ectopic, heterologous, or illegitimate transformants express both a negative and a positive selection marker.

3. The method of claim 1, wherein said cell is a fungal cell.

4. The method of claim 3, wherein said fungal cell comprises mycelial fragments, spores, and protoplasts.

5. The method of claim 1, wherein said negative selection marker confers susceptibility to an agent.

6. The method of claim 5, wherein said negative selection marker is operably linked to a promoter sequence.

7. The method of claim 5, wherein said negative selection marker is selected from the group consisting of a herpes simplex virus thymidine kinase (HSVtk), and a bacterial endotoxin gene.

8. The method of claim 7, wherein said negative selection marker is HSVtk.

9. The method of claim 1, wherein said positive selection marker confers resistance to an antibiotic.

10. The method of claim 9, wherein said positive selection marker is selected from the group consisting of hygromycin B phosphotransferase (hph) gene, neomycin phosphotransferase (npt) gene, mutated beta-tublin (ben) gene, Bar, Ble, Sat-1, and cbx.

11. The method of claim 10, wherein said positive selection marker is a hygromycin resistance gene (hph).

12. The method of claim 3, wherein said fungal cell is a fungal species selected from the group consisting of *Aspergillus fumigatus*, *Botrytis cineria*, *Magnaporthe grisea* and *Fusarium oxysporum*.

13. The method of claim 12, wherein said fungal cell is *Magnaporthe grisea*.

14. The method of claim 12, wherein said fungal cell is *Fusarium oxysporum*.

15. The method of claim 1, wherein said transformation is mediated by *Agrobacterium tumefaciens*.

16. A strain of fungal cells transformed by the method of claim 1.

17. A polynucleotide construct comprising a first polynucleotide sequence encoding a negative selection marker linked to a fragment of DNA flanked by DNA sequences homologous to a polynucleotide to be targeted, wherein said DNA fragment is disrupted by a positive selection marker.

18. A vector comprising the polynucleotide construct of claim 17.

19. The vector of claim 18 capable of transforming fungal cells in culture susceptible to infection by *Agrobacterium tumefaciens*.

20. An *Agrobacterium tumefaciens* cell comprising the vector of claim 18.

21. A method of identifying a gene knockout mutant comprising:

(a) providing a polynucleotide construct comprising a first polynucleotide sequence that encodes a negative selection marker linked to a fragment of DNA flanked by DNA sequences homologous to the polynucleotide to be targeted, wherein said DNA fragment is disrupted by a positive selection marker;

(b) introducing into *Agrobacterium* the construct provided in (a), thereby producing a resultant *Agrobacterium* cells containing a DNA fragment with a disrupted sequence;

(c) incubating *Agrobacterium* produced in (b) with fungal cells under conditions so that T-DNA containing said construct is integrated into a fungal cell genome, wherein transformants resulting from knockout lack a negative selection marker and ectopic, heterologous, or illegitimate transformants express both a negative and a positive selection marker; and

(d) selecting knockout mutants by subjecting transformed fungal cells to a positive and a negative selection agent.

22. The method of claim 21, wherein said DNA fragment is a gene of interest that is rendered nonfunctional by insertion of a selection marker, thereby generating a null mutation to assess a phenotypic affect of at least one mutant allele.

23. The method of claim 21, wherein said fungal cells comprise mycelial fragments, spores, and protoplasts.

24. The method of claim 21, wherein said negative selection marker is operably linked to a promoter sequence.

25. The method of claim 21, wherein said positive selection marker is selected from the group consisting of hygromycin B phosphotransferase (hph) gene, neomycin phosphotransferase (npt) gene, mutated beta-tublin (ben) gene, Bar, Ble, Sat-1, and cbx.

26. The method of claim 25, wherein said positive selection marker is a hygromycin resistance gene.

27. The method of claim 21, wherein said negative selection marker is selected from the group consisting of herpes simplex virus thymidine kinase (HSVtk), a bacterial endotoxin gene, and a diphtheria toxin A fragment.

28. The method of claim 27, wherein said negative selection marker is HSVtk.

29. The method of claim 21, wherein said negative selection agent is selected from the group consisting of ganciclovir, acyclovir, and 5-fluoro-2'-deoxyuridine (F2dU).

30. The method of claim 29, wherein said negative selection agent is 5-fluoro-2'-deoxyuridine (F2dU).

31. The method of claim 21, wherein said positive selection agent is selected from the group consisting of hygromycin B, geneticin or G-418, benomyl, basta, phleomycin, nourseothricin, and carboxin.

32. The method of claim 31, wherein said positive selection agent is hygromycin B.

33. The method of claim 21, wherein said fungal cells are fungal species selected from the group consisting of *Aspergillus fumigatus*, *Botrytis cineria*, *Magnaporthe grisea* and *Fusarium oxysporum*.

34. The method of claim 33, wherein said fungal cells are *Magnaporthe grisea*.

35. The method of claim 33, wherein said fungal cells are *Fusarium oxysporum.*

36. A strain of fungal cells transformed by the method of claim 21.

37. A method of transforming fungal cells to identify mutants comprising:

inserting a polynucleotide construct to be introduced into fungal cells into an *Agrobacterium*-based vector between T-DNA borders in that vector;

introducing said vector containing said DNA construct into *Agrobacterium tumefaciens* cells, wherein said cells contain a virulence region in its DNA;

inducing virulence genes to T-DNA containing said construct from said *Agrobacterium tumefaciens* and incubating said *Agrobacterium tumefaciens* with a fungal cells to be transformed; and

selecting transformed fungal cells from untransformed fungal cells by subjecting transformants to a positive and a negative selection agent.

38. The method of claim 37, wherein said fungal cells comprise mycelial fragments, spores, and protoplasts.

39. The method of claim 37, wherein said polynucleotide construct comprises a disruption cassette.

40. The method of claim 39, wherein said cassette comprises a DNA fragment having at least one mutant allele, wherein said mutant allele is generated by the insertion of a positive selection marker.

41. The method of claim 37, wherein said construct further comprises a negative selection marker that is operably linked to a promoter sequence.

42. The method of claim 40, wherein said positive selection marker is selected from the group consisting of hygromycin B phosphotransferase (hph) gene, neomycin phosphotransferase (npt) gene, mutated beta-tublin (ben) gene, Bar, Ble, Sat-1, and cbx.

43. The method of claim 42, wherein said positive selection marker is a hygromycin resistance gene.

44. The method of claim 37, wherein said negative selection marker is selected from the group consisting of herpes simplex virus thymidine kinase (HSVtk), a bacterial endotoxin gene, and a diphtheria toxin A fragment.

45. The method of claim 44, wherein said negative selection marker is HSVtk.

46. The method of claim 37, wherein said negative selection agent is selected from the group consisting of ganciclovir, acyclovir, and 5-fluoro-2'-deoxyuridine (F2dU).

47. The method of claim 46, wherein said negative selection agent is 5-fluoro-2'-deoxyuridine (F2dU).

48. The method of claim 32, wherein said positive selection agent is selected from the group consisting of hygromycin B, geneticin or G-418, benomyl, basta, phleomycin, nourseothricin, and carboxin.

49. The method of claim 48, wherein said positive selection agent is hygromycin B.

50. The method of claim 37, wherein said fungal cells are fungal species selected from the group consisting of *Aspergillus fumigatus*, *Botrytis cineria*, *Magnaporthe grisea* and *Fusarium oxysporum*.

51. The method of claim 50, wherein said fungal cells are *Magnaporthe grisea*.

52. The method of claim 50, wherein said fungal cells are *Fusarium oxysporum*.

53. A strain of fungal cells transformed by the method of claim 37.

54. A method of identifying and selecting transformants comprising:

transforming fungal cells with *Agrobacterium tumefaciens* under suitable conditions whereby recombination occurs, wherein transformants resulting from a gene knockout lack a negative selection marker and ectopic, heterologous, or illegitimate transformants will express a negative and a positive selection marker, said *Agrobacterium tumefaciens* comprising a gene disruption vector, said vector comprises a polynucleotide encoding a negative selection marker linked to a fragment of DNA flanked by DNA sequences homologous to the polynucleotide to be targeted, wherein said fragment contains at least one mutant allele, wherein said mutant allele is generated by the insertion of a positive selection marker;

regenerating transformants in the presence of both a positive and a negative selection agent; and

selecting putative knockout mutants.

55. The method of claim 54, wherein said fungal cells comprise mycelial fragments, spores, and protoplasts.

56. The method of claim 54, wherein said fungal cells are fungal species selected from the group consisting of *Aspergillus fumigatus*, *Botrytis cineria*, *Magnaporthe grisea* and *Fusarium oxysporum*.

57. The method of claim 56, wherein said fungal cells are *Magnaporthe grisea*.

58. The method of claim 56, wherein said fungal cells are *Fusarium oxysporum*.

59. A strain of fungal cells transformed by the method of claim 54.

60. A method of identifying and selecting transformants comprising:

transforming fungal cells with *Agrobacterium tumefaciens* cells under suitable conditions

whereby recombination occurs wherein transformants resulting from gene knockout lack a negative selection marker and ectopic, heterologous, or illegitimate transformants express both a negative and a positive marker, said *Agrobacterium tumefaciens* cells comprising a gene disruption vector, said vector comprising in an operable orientation a pGreen II cloning site, a polynucleotide sequence that encodes a negative selection marker, said sequence is linked to a fragment of DNA, wherein said DNA fragment is disrupted by a positive selection marker; and

selecting gene knockout mutants by subjecting transformed fungal cells to a positive and a negative selection agent.

61. The method of claim 60, wherein said fungal cells are fungal species selected from the group consisting of *Magnaporthe grisea* and *Fusarium oxysporum.*

62. A targeted polynucleotide having undergone homologous recombination with the vector of claim 1 so as to incorporate said DNA fragment disrupted by a positive selectable marker into said targeted polynucleotide.

63. A polynucleotide construct in an operable orientation comprising a first polynucleotide sequence encoding a negative selection marker; a DNA fragment disrupted by a positive selection marker; and a pGreen II cloning site.

64. The polynucleotide construct of claim 17, wherein said first polynucleotide sequence a herpes simplex virus thymidine kinase (HSVtk) and said second polynucleotide sequence disrupted by an hygromycin resistance selection marker.

65. The polynucleotide construct of claim 17, wherein said second polynucleotide is homologous to a targeted polynucleotide sequence in a fungal host cell.